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In vivo and in vitro Labelling of Epithelial Tumor Cells with Anti 17-1A Monoclonal Antibodies in Bone Marrow of Cancer Patients

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INTRODUCTION

Only in the minority of patients with solid tumors can distant metastases be detected when the diagnosis of the primary tumor is made. And yet even after highly efficient locoregional removal of the primary cancer a majority of those patients develops distant metastases in the course of several years. Thus it has to be assumed that systemic distribution takes place in the majority of patients at a rather early stage in their tumor growth, most probably prior to the time of operation on the primary tumor.

The detection of such early micrometastases or disseminated tumor cells poses a problem for conventional diagnostic procedures. The development of new immunologic techniques has opened new prospectives for this difficult field. In particular immunocytochemical procedures permit the unequivocal identification of single tumor cells in lymph node tissue or bone marrow.(1) As to the therapy of micrometastases, adjuvant cytostatic or hormonal regimens are only of limited benefit for those patients with the most frequent organ tumors. It is this difficult therapeutic domain where passively administered antibodies are supposed to have their greatest efficiency, since antibody-mediated lysis is effective on nonproliferating cells and works best on single cells. Since the monoclonal antbody 17-1A had already been introduced into the clinic in phase I and II trials, we have investigated the use of this antibody in a diagnostic and therapeutic approach in patients with high risk of micrometastasis of various epithelial tumors.(2,3)

PATIENTS, MATERIALS AND METHODS

In 73 patients with primary cancer of the breast (n = 38), colon or rectum (n = 24), stomach (n = 7) or lung (n = 4), bone marrow aspirations were performed during resection of the tumor. After primary diagnosis all patients underwent an extensive diagnostic

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program which included chest x-ray, sonography of the upper abdomen, bone scintigraphy and abdominal computertomography. Patients hospitalized for other than malignant diseases served as controls. Informed consent was obtained from all participating patients. Staging of patients was performed according to the TNM classification.

Bone marrow was aspirated from the iliac crest and, less often, from the sternum. A mean volume of 8.5 ml of marrow per patient was obtained which yielded an average of 1.6×10^8 nucleated cells. After density centrifugation in Ficoll-Hypaque the cells were centrifuged on glass slides with a cytocentrifuge. After fixation with acetone the cells were stored at -70°C. For immunocytochemistry the antibody MAB anti-cytokeratin Ck2 (IgG_1) was used, which reacts with cytokeratin component 18 of cylindrical epithelia.[4,5] The antibodies M77 (IgG_{2b}) and M79 (IgG_{2a}), recognizing the 17-1A antigen, were used in a 1:1 mixture to detect the 17-1A glycoprotein (37 kD) on the cell membrane of epithelial tumor cells.[6] The antibody reaction was developed with the alkaline phosphatase (AP) technique using a polyvalent rabbit-anti-mouse Ig antiserum and preformed complexes of AP and monoclonal anti-AP antibodies.[7] For detection of cells labelled in vivo with anti-17-1A MAB, the fixed marrow cells were stained without prior incubation with the first antibody. Giemsa staining was performed in parallel on all marrow aspirated.

Therapy with MAB 17-1A

For therapy MAB 17-1A was kindly provided by Dr. Hilary Koprowski, Philadelphia. After 1 hr centrifugation at 100,000 g, 100 or 500 mg of 17-1A was diluted in 250 ml saline and infused i.v. over a period of 1-2 hours. Patients were treated according to a study protocol approved by the Ethics Committee of the University of Munich Medical Faculty (to be published elsewhere).

RESULTS

Detection of isolated tumor cells in bone marrow aspirates

After staining with anti-cytokeratin antibody Ck2 epithelial tumor cells were observed in 16 out of 73 aspirates (Table 1). In 11 out of the 16 Ck2-positive marrows the combination of two anti 17-1A antibodies (M77 and M79) clearly stained epithelial cells (Table 2). Examination of the marrow after conventional Giemsa staining led to the diagnosis of tumor cells in only 4 patients, all of whom were stage M_1, i.e. had manifest distant metastases.

Marrow aspirates of control patients with no malignant disease gave no positive reaction with anti-cytokeratin in 60 cases and likewise no positive reaction with the two anti-17-1A antibodies (M77 and M79) in 8 cases.

In vivo labelling of tumor cells in marrow after infusion with MAB 17-1A

Six patients in whom Ck-2 positive cells were detected in the bone marrow were selected for infusion therapy with MAB 17-1A (Table 3). Of these patients, three showed tumor cells in the marrow stained with the combination of M77 and M79 immediately prior to infusion. One of the 3 patients also had clinically manifest metastases. In the other two patients mouse Ig-coated tumor cells were detected 2 hr after infusion without in vitro addition of MAB 17-1A. Thus, the infused mouse IgG_{2a} must have labelled the cells in vivo.

In patient W.L., suffering from wide-spread metastatic mammary cancer, in vivo labelling of tumor cells in marrow was not detected.

Table 1

Detection of isola
aspirates with mc

Histologic diagnosis of patients	
Breast cancer (n = 38	
	Stage M_0
	Stage M_1
Colo-rectal cancer (n	
	Stage M_0
	Stage M_1
Gastric cancer (n = 7	
	Stage M_0
	Stage M_1
Bronchial cancer (n =	
	Stage M_0
	Stage M_1
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and/or operated	
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* APAAP method as out

Monitoring tumor cells

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Table 1

Detection of isolated epithelial tumor cells in bone marrow aspirates with monoclonal anti-cytokeratin antibody (Ck2)

Histologic diagnosis of patients	Positive Reactions MAB Ck2*
Breast cancer (n = 38)	
Stage M_0	5 / 35
Stage M_1	3 / 3
Colo-rectal cancer (n = 24)	
Stage M_0	2 / 15
Stage M_1	2 / 9
Gastric cancer (n = 7)	
Stage M_0	0 / 4
Stage M_1	3 / 3
Bronchial cancer (n = 4)	
Stage M_0	0 / 3
Stage M_1	1 / 1
total cancer patients	16 / 73
control patients hospitalized and/or operated for non-malignant disease	0 / 60

* APAAP method as outlined under METHODS

Monitoring tumor cells in patients treated with MAB 17-1A

According to the above mentioned protocol, 3 patients were treated with 17-1A infusions in monthly intervals (Table 4). When analyzed by immunocytochemistry 2 patients (P.A., B.H.) had Ck-2 positive tumor cells in their marrow samples, which became negative during therapy. In patient P.A., however, distinct Ck-2-positive tumor cells were found 6 weeks after termination of the antibody therapy. In the third patient (R.M.), where monitoring by repeated marrow aspiration was attempted, no postive tumor cells could be detected during therapy.

Table 2

Correlation between anti-cytokeratin (Ck2) positive and anti-17-1A-positive tumor cells in bone marrow of patients with various forms of cancer

	Positive staining with anti-cytokeratin	Staining with anti-17-1A positive	negative
Breast cancer	8	5	3
Colo-rectal cancer	4	3	1
Gastric cancer	3	2	1
Bronchial cancer	1	1	0
Total	16	11	5

DISCUSSION

At the time of diagnosis a high percentage of patients with the most frequent solid tumors already has widespread micrometastases. The detection of isolated disseminated tumor cells has been distinctly improved since the introduction of monoclonal antibodies for immunocytochemical analysis. Using a polyclonal antisera against an epithelial membrane antigen (EMA), Neville and coworkers already had detected micrometastases and isolated tumor cells in marrow of mammary carcinoma with a surprisingly high frequency.[8,9] The significance of their findings was weakened, however, by the subsequent demonstration of anti-EMA antibodies cross-reacting with lymphoid cells[10]. Thus the use of monoclonal antibodies against cytokeratin appeared of great advantage since anti-cytokeratin was consistently negative on bone marrow cells of normal donors.[10,11] A preliminary study of ours had shown the usefulness of the anti-Ck2 antibody when combined with the APAAP method, where alkaline phosphatase staining gave a bright red color with remarkably low background.[12]

Thus in the present study 7 out of 57 patients without clinically manifest distant metastases carried epithelial tumor cells in their marrow. The majority of the Ck2-positive patients were also found to be positive after staining with MAB 17-1A. This antibody deserved special attention because of its unusual homogeneous staining of tumors in situ and its lack of side effects in a large group of patients. The in vitro cytotoxicity of this antibody in an ADCC system[13] is supported by its in vivo inhibitory activity on tumor transplants in the nude mouse.[14]

Though the vast majority of patients in phase I and II trials has been treated with MAB 17-1A in an advanced stage of their disease, objective responses have been reported.[2,3] It seems therefore warranted to expore the therapeutic efficiency of the MAB 17-1A in patients without overt distant metastases. Though tumor-free interval or survival will be the most significant parameter in such a regimen, detection of isolated tumor cells and their monitoring under therapy may be a valuble adjunct for improvement of therapeutic strategies. Metastasis like carcinogenesis is a multistep pro-

in (Ck2) positive and

one marrow of patients

cancer

Staining with anti-17-1A positive	negative
5	3
3	1
2	1
1	0
11	5

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Table 3

In vivo labelling of tumor cells in bone marrow after infusion of 17-1A antibody

Patient	Diagnosis	Stage	Dose of 17-1A MAB infused (mg)	Time interval of marrow aspiration after MAB infusion	In vitro Labelling prior to infusion Ck2	In vitro Labelling prior to infusion 17-1A	In vivo labelling after infusion
B.H.	Colon cancer	$T_3N_2M_0$	500	2 hr	+	-	-
				4 days	+		-
K.A.	Colon cancer	$T_3N_1M_0$	500	2 hr	+	+	+
P.A.	Breast cancer	$T_2N_1M_0$	100	2 hr	+	+	+
S.B.	Breast cancer	$T_2N_1M_0$	100	2 hr	+	-	-
W.L.	Breast cancer	$T_3N_2M_1$	100	4 hr	+	+	-
T.F.	Breast cancer	$T_2N_2M_1$	100	18 hr	+	-	-
				8 days	+		-

Table 4

Monitoring of tumor cells in bone marrow of patients treated with MAB 17-1A in an adjuvant fashion

Patient	Diagnosis	Stage	Date	Dose (mg)	In vitro labelling with Ck2
P.A.	Breast cancer	$T_2N_1M_0$	11.06.85	100	+
			9.07.85	100	-
			6.08.85	100	-
			19.09.85	100	-
			31.10.85	-	+
B.H.	Colon cancer	$T_3N_2M_0$	1.10.85	500	+
			31.10.85	100	+
			27.11.85	100	-
			30.12.85	100	-
			28.01.86	100	-
R.M.	Colon cancer	$T_2N_2M_0$	29.11.85	500	-
			23.12.85	100	-
			28.01.86	100	-
			24.02.86	100	-
			21.03.86	100	-

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cess, thus invasiveness and dissemination are necessary steps but not sufficient to lead to successful metastasis. Further studies are required to analyze biological qualities of these disseminated tumor cells and to correlate those to the long term course of the disease.

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